Example 6

Effect of Non-Aerobic Conditions on Refolding Efficiency

FIGS. **2** and **3** demonstrate that when the thiol-pair buffer strength is selected appropriately, taking into account the protein concentration and number of cysteine residues in the protein, the sensitivity to external influences, such as oxygen, is significantly reduced. This allows for a non-aerobic refolding condition that is significantly easier to transfer between scales and reactor configurations.

FIG. **2** compares the RP-HPLC analytical species distribution between a 15 L-scale refold and a 20 mL-scale refold under several environmental conditions. For Condition 1 (the trace labeled "1" in FIG. **1**), the solubilization chemicals and solutions were dispensed in air and the refold mixture was incubated in air. In Condition 2 solubilization chemicals and solutions were dispensed in air and incubated under nitrogen headspace. In Conditions 3-7 solubilization chemicals and solutions were dispensed under nitrogen overlay conditions and in conditions 3, 5, 6, and 7 solubilization chemicals and solutions were incubated under nitrogen. In Condition 7, the refold solution was also stripped of nitrogen prior to combination with the solubilization solution. In Condition 4 the solubilization chemicals and solutions were incubated under ambient air conditions.

The results shown in FIG. **2** demonstrate that the conditions under which the solubilization chemicals and solutions were dispensed or incubated in the presence of air (i.e., Conditions 1, 2, and 4) do not achieve results that are comparable to the larger-scale control. In Conditions 1, 2 and 4, increased formation of oxidized species (pre-peaks) are observed. The pre-peaks are indicated by arrows in the panels for Conditions 1, 2 and 4.

FIG. **3** compares the RP-HPLC analytical results of an identified condition, achieved as described in Example 2, at 1 L-scale and 2000 L-scale. In this figure, essentially no difference in the distribution of species is detectable. Taken together, FIGS. **2** and **3** demonstrate that when aeration is carefully controlled, the small-scale refold reactions are more predictive of those expected upon scale-up of the refold reaction, facilitating the implementation of large-scale protein refolding processes.

What is claimed is:

1. A method of refolding a protein expressed in a non-mammalian expression system and present in a volume at a concentration of 2.0 g/L or greater comprising:

(a) contacting the protein with a refold buffer comprising a redox component comprising a final thiol-pair ratio having a range of 0.001 to 100 and a redox buffer strength of 2 mM or greater and one or more of:

(i) a denaturant;

(ii) an aggregation suppressor; and

(iii) a protein stabilizer;

to form a refold mixture;

(b) incubating the refold mixture; and

(c) isolating the protein from the refold mixture.

2. The method of claim **1**, wherein the final thiol-pair ratio is selected from the group consisting of 0.05 to 50, 0.1 to 50, 0.25 to 50, 0.5 to 50, 0.75 to 40, 1.0 to 50 and 1.5 to 50, 2 to 50, 5 to 50, 10 to 50, 15 to 50, 20 to 50, 30 to 50 or 40 to 50.

3. The method of claim **1**, wherein the thiol-pair buffer strength is selected from the group consisting of greater than or equal to 2.25 mM, 2.5 mM, 2.75 mM, 3 mM, 5 mM, 7.5 mM, 10 mM and 15 mM.

4. The method of claim **1**, wherein the protein is present in the volume in a non-native limited solubility form.

5. The method of claim **4**, wherein the non-native limited solubility form is an inclusion body.

6. The method of claim **1**, wherein the protein is present in the volume in a soluble form.

7. The method of claim **1**, wherein the protein is recombinant.

8. The method of claim **1**, wherein the protein is an endogenous protein.

9. The method of claim **1**, wherein the protein is an antibody.

10. The method of claim **1**, wherein the protein is a complex protein.

11. The method of claim **1**, wherein the protein is a multimeric protein.

12. The method of claim **1**, wherein the protein is an Fc-protein conjugate.

13. The method of claim **1**, wherein the non-mammalian expression system is one of a bacterial expression system and a yeast expression system.

14. The method of claim **1**, wherein the denaturant is selected from the group consisting of urea, guanidinium salts, dimethyl urea, methylurea and ethylurea.

15. The method of claim **1**, wherein the protein stabilizer is selected from the group consisting of arginine, proline, polyethylene glycols, non-ionic surfactants, ionic surfactants, polyhydric alcohols, glycerol, sucrose, sorbitol, glucose, Tris, sodium sulfate, potassium sulfate and osmolytes.

16. The method of claim **1**, wherein the aggregation suppressor is selected from the group consisting of arginine, proline, polyethylene glycols, non-ionic surfactants, ionic surfactants, polyhydric alcohols, glycerol, sucrose, sorbitol, glucose, Tris, sodium sulfate, potassium sulfate and osmolytes.

17. The method of claim **1**, wherein the thiol-pairs comprise at least one component selected from the group consisting of glutathione-reduced, glutathione-oxidized, cysteine, cystine, cysteamine, cystamine and beta-mercaptoethanol.

18. The method of claim **1**, wherein the incubation is performed under non-aerobic conditions.

19. The method of claim **1**, wherein the isolation comprises contacting the mixture with an affinity separation matrix.

20. The method of claim **19**, wherein the affinity separation matrix is a Protein A resin.

21. The method of claim **19**, wherein the affinity resin is a mixed mode separation matrix.

22. The method of claim **1**, wherein the isolating comprises contacting the mixture with an ion exchange separation matrix.

23. The method of claim **1**, wherein the isolating further comprises a filtration step.

24. The method of claim **23**, wherein the filtration step comprises depth filtration.

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